CONTINUOUS FERMENTATION STUDIES

BY

ROBERT ELWOOD WILSON

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B. S., University of Illinois, 1950
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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT of the REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMICAL ENGINEERING IN THE GRADUATE COLLEGE
OF THE UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS
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INTRODUCTION

This study of continuous growth or fermentation had as its goal to better understand and describe continuous fermentation and if possible to predict, somewhat, results which might be expected by employing continuous fermentation.

This work was divided into three main parts.

1. Derivation and verification of a generalized equation to describe continuous fermentation.
2. Studies of a cycling phenomenon which is exhibited under certain conditions during continuous fermentation.
3. A comparison of morphological changes taking place in continued vegetative transfer growth as opposed to continuous growth.

A rather simple but effective type of laboratory apparatus is described which was designed and constructed for the above studies.

Novick and Szilard (4) in 1950 carried out studies on the spontaneous mutation of bacteria using a piece of apparatus which they called a "chemostat". A means was provided to add fresh nutrient to the growth vessel at a constant rate while an equal amount was removed from the growth vessel by simple overflow. For consistent results when using this type of equipment, the entire system must be thermostated. In this type of apparatus it was possible to maintain a constant population density over an indefinite length of time.

Adams and Hungate (1) in 1950 grew yeast continuously on fruit juice wastes. Their main interest was in developing a method to predict continuous fermentation cycle time from analysis of the batch growth curve.

Monod (3) in 1950 also carried out continuous growth experiments in which he was interested in studying the growth of organisms on media which contained minimal or sub-minimal amounts of certain required growth factors. He also derived a general equation to describe continuous fermentation.

Maxon and Johnson (2) in 1950 made a study in which they carried out a continuous growth of yeast as one part of this study. During this study they encountered an unusual cycling phenomenon with **Saccharomyces cerevisiae.** This cycling did not manifest itself at all times but apparently occurred only when the system was in a state of unbalance. When the cycling did occur it appeared in the population density versus time, and carbon dioxide evolution versus time.

EQUIPMENT

The continuous growth equipment used in this study was comprised of the following pieces.

1. Fermentation or growth vessel.
2. Constant flow pump.
3. Media supply bottles.
4. Miscellaneous auxiliary equipment.

The fermentation or growth vessel was constructed from a 500 ml. gas scrubbing bottle with a sintered glass sparger at the bottom, a capillary overflow, an air outlet port, an inoculation port, and a fitted cover. The cover contained three glass tubes through which liquid substances could be pumped into the growth vessel.

The constant flow pump was a Sigmamotor pump coupled to a 1/4 horsepower motor by a 300-1 reduction gear.

The media supply bottles were 4 liter bottles with fitted stoppers.

The miscellaneous equipment consisted of glass wool air filters, a constant temperature water bath, and manometers.

EXPERIMENTAL

The organisms used in this study were **Pseudomonas fluorescens** and **Bacterium linens** for the verification of the general derived equation, **Saccharomyces carlsbergensis** in the cycling experiments, and **Streptomyces griseus** in the last series of experiments comparing continued vegetative transfer growth and continuous growth.

The following equation was derived:

$$dN / dt = N (\ln 2 / tg - 1 / tr)$$

where

N — total number of organisms.
t — time.
tg — time of generation.
tr — time of retention.

This equation was verified for the following conditions:

1. The time of generation approaching infinity.
2. The time of retention approaching infinity.
3. $(\ln 2 / tg - 1 / tr) = 0$
4. Intermediate values in which $(\ln 2 / tg < \text{or} > 1 / tr)$.

The cycling experiments carried out were the following:

1. Measurements of the time of generation as a function of pH were made.
2. The pH and population density versus time were observed during cycling runs.
3. Attempts were made to induce cycling by using media with deficient sugar concentrations.

The experiments which were carried out to compare the morphological changes taking place in continuous vegetative transfer growth as compared with continuous growth were the following. **Streptomyces griseus** was introduced into both a continuous growth vessel and a batch type vessel simultaneously. Growth proceeded in both vessels and photomicrographs were made of the organisms in both vessels at zero days, three days, six days, and eight days. Transfers were made from the batch vessel to another batch vessel where fresh nutrient was provided at three and six days.

CONCLUSIONS

A General Equation to describe continuous growth was derived and verified for several limiting and intermediate conditions for non-cycling systems.

Cycling growth was induced by decreasing the rate of inflow of fresh nutrient to a value slightly below the value necessary to maintain a constant population density. The relationship between the pH an population density, which exists during cycling was demonstrated. It also was shown that the use of sugar deficient medium does not in itself induce cycling.

Photomicrographs of **Streptomyces griseus** indicated that no morphological changes of the organism occurred when it was grown continuously, but that it changed very markedly when grown by the use of continued vegetative transfers. The results suggest that the continuous growth of microorganisms is a practical technique and that the kinetics of continuous growth may be predicted from the mathematical relationships outlined here.

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VITA

The author was born in Decatur, Illinois on July 2, 1926. He attended the public schools of Decatur, Illinois, graduating from Decatur High School in 1944. He then served in the United States Navy from 1944 to 1946, spending one year as a radio-technician and one year in the V-12 and NROTC. He attended Baldwin-Wallace College and the University of Michigan while in the Navy. In 1946 he enrolled at the University of Illinois where he received a Bachelor of Arts degree with a major in Chemistry in 1948 and a Bachelor of Science degree in Chemical Engineering in 1949. He entered the University of Minnesota in 1949 and received a Master of Science degree in Chemical Engineering in 1950. In the fall of 1950 he re-entered the University of Illinois to work toward the PhD. The author held teaching assistantships at both the University of Illinois and the University of Minnesota. He is a member of Tau Beta Pi, Sigma Tau, Chi Gamma Iota, and Phi Lambda Upsilon.